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The Potential of Semiochemicals for Control of *Phorodon humuli* (Homoptera: Aphididae)†

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Abstract: Field experiments employing yellow water-traps with vials releasing methyl salicylate, butyl isothiocyanate, 4-pentenyl isothiocyanate and diethyltoluamide were conducted during the spring migration of *Phorodon humuli* (Schrank), with the aim of identifying substances which might be used in the field to deter landing on hop plants. Methyl salicylate and the two isothiocyanates reduced trap catches of *P. humuli*. During the spring of 1994 a slow-release formulation of methyl salicylate and a β-acid-rich hop resin sprayed on to hop plants did not reduce aphid infestations significantly. In autumn *cis,cis*-nepetalactol, the main component of *P. humuli*'s sex pheromone, prepared by various synthetic routes, increased trap catches of males and gynoparae equally. Catches of males in pheromone traps situated in a hop garden decreased with increasing trap height. Catches of males in traps charged with increasing doses of the *cis,cis*-nepetalactol peaked at 1 mg and then plateaued, whereas catches of gynoparae peaked similarly at 1 mg and then decreased. The effects of kairomones from an extract of the primary host, sex pheromone and a visual cue from yellow compared with clear water-traps were additive. The prospects for developing a semiochemicals-based control strategy against *P. humuli*, using some or all of the above elements, are discussed.

Key words: allomones, aphid, field experiments, Homoptera, hops, kairomones, pheromones, *Phorodon humuli*, repellents

1 INTRODUCTION

The damson-hop aphid *Phorodon humuli* (Schrank) (Homoptera: Aphididae) is the single most important insect pest of hops and occurs to a greater or lesser extent in most hop-growing areas of the world. It is an holocyclic/heteroecious species with several *Prunus* spp. (Rosaceae) as primary (winter) hosts and the hop *Humulus lupulus* L. (Cannabaceae) as the sole secondary host.[1] Two migratory flights are undertaken during the year, coinciding roughly with the beginning and end of vegetative growth of the hops. The behaviour involved in location of the host plants during these migratory flights is thought to be controlled by olfactory chemical stimuli emitted by the host plants, as well as by such

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visual cues as colour and shape. During the autumn migration sex pheromone cues are also involved. A role for semiochemicals in host location and recognition by migrating damson-hop aphids has been shown for both the flight from primary to secondary host and the return migration.[2,3]

1.1 Stimulo-deterrent diversionary (push-pull) strategy against spring migrants

Pickett, Wadhams and Woodcock[4] proposed the simultaneous use of 'push' (i.e. repellent) and 'pull' (i.e. attractant) semiochemicals as the basis for integrated crop-protection strategies, to be used in conjunction with selective insecticides or biological agents to control pest-insect populations. The repellent effects of volatiles from non-host plants have been described for various aphid species[5–8] and it has been suggested that application of such materials to the host plant may be used as part of a push–pull strategy to mask its attractiveness. Analysis of the headspace from hops infested with *Phorodon humuli* has shown that this contains volatile components that may repel aphids (allomones) as well as those that they find attractive (kairomones).[3] As the overall attractiveness of the hops to *P. humuli* may be attributable in part to the relatively greater abundance of kairomonal components in the headspace, an augmentation in the proportion of hop allomones might be sufficient to mask, or at least reduce, the attractiveness of the hops. Equally, headspace components of hops with kairomonal properties might be used to steer flying damson-hop aphids to selected areas, either in the form of a trap, or a strip of trap crop at the edge of a hop garden, where selective population-reducing measures could be applied.

1.2 Semiochemicals involved in the autumn migration

Campbell *et al.*[2] demonstrated both in laboratory and in field studies that male *P. humuli* are influenced strongly by pheromonal cues emitted by oviparous females present on the primary host. Traps fitted with sources containing *cis,cis*-nepetalactol, the main component of the pheromone, caught substantial numbers of *P. humuli* males. Results of a further field study[9] agreed with this finding, confirming that *cis,cis*-nepetalactol was also attractive to gynoparae,[10] and found that the attractiveness of the sex pheromone to both sexes was enhanced by the simultaneous release of a variety of primary-host volatiles.

1.3 Aims of the study

The principal aim of experiments conducted during the spring migration was to identify host-plant stimuli with an attractive effect on alate *P. humuli*. A further aim was to test, under field conditions, constituents selected from both non-host and host plants which had shown allomone activity in the laboratory. The potential for such substances as part of a stimulo-deterrent strategy against the damson-hop aphid is discussed. The main aim of experiments conducted in the autumn was to examine the interaction between a visual cue, volatile primary-host kairomones and the sex pheromone. Tests were also carried out to compare the attractiveness of sex pheromone prepared by different chemical synthesis routes and to determine the effects of pheromone concentration on the catch of *P. humuli*. The ultimate aim of this part of the study was the optimisation of an attractant system for use either in mass-trapping or 'lure-and-kill' approaches to reduce numbers of the overwintering egg-stage of the damson-hop aphid by trapping the autumn migrants as they leave the hops.

2 MATERIALS AND METHODS

2.1 Field sites

Experiments were conducted at three sites, Pfab, Gschwend and Barthhof, in the Holledau region of Bavaria during the spring/early summer of 1993 and 1994, and in the autumn of 1992 and 1993. Trapping experiments with spring migrants were carried out at the edge of the Pfab site, a commercial hop garden of 1 ha planted with cv. Perle. In 1994 a slow-release rope-formulation of methyl salicylate and an aqueous spray of β-acid-rich hop-resin were applied to hops (cv. Hersbrucker Spät) in two separate plots, each of 30 plants, at the Gschwend site. Experiments with autumn migrants were carried out at the Pfab and Barthof sites, the latter a commercial hop garden of around 2 ha planted with cv. Magnum. Unless stated otherwise, traps (see below) were set up 10 m apart in randomised plots between the rows of hop plants. Traps were emptied and their positions re-randomised daily.

2.1.1 *Traps*

The design of water-filled traps was based on that described by Campbell *et al.*[2] They consisted of clear plastic Petri dishes (14·5 cm diameter) which were either left unpainted or were painted on the outside with two coats of canary-yellow paint (ICI autocolor P030-0409). Traps were filled with water containing a trace of detergent, and were mounted on 1-m-high wooden stakes. The dispenser for test odours consisted of a 1-ml Chromacol vial (Type: 08-CPV) with a 1-mm hole drilled in its lid and was attached over the centre of the trap.

2.2 Experiments with potential repellents for spring migrants

Samples of methyl salicylate (**A**) butyl isothiocyanate (**B**) and *N,N*-diethyltoluamide (**D**) were obtained from

Aldrich Chemical Co. 4-Pentenyl isothiocyanate (**C**) was synthesised in two steps starting from 5-bromo-1-pentene; reaction with sodium thiocyanate yielded 1-pentenyl thiocyanate which isomerised in the presence of potassium iodide and calcium carbonate giving the required 4-pentenyl isothiocyanate. Traps were fitted with dispensers containing either the test material (20 mg) or no volatiles (control).

For the field-plot experiments, a slow-release 'PVC-rope' formulation containing methyl salicylate (30 ml litre^{-1}; prepared by AgriSense-BCS) was used. The rope-formulation was tied to the top wire of the hop-trellis at the point where the guide wire for the hops was attached, and was wrapped loosely around this wire and the young hop plants seven or eight times before being tied to the attachment point of the wire at the base of the plant. One rope was attached to each hop plant at the start of the *P. humuli* flight on 19 May 1994. The treatment was repeated with fresh lengths of the methyl salicylate-containing rope two weeks later. For the β-acid treatment the hop foliage was sprayed, using a hand-held sprayer (Gloria 172 RTG), with a solution of a commercial hop resin (100 g) in ethanol (500 ml) containing 'Renex 36' wetting agent (20 ml) and diluted with tap water (5 litre): the resin, obtained from Scottish and Newcastle Breweries, UK, contained *c*.50% β-acids. For each treatment, the hop plants were sprayed twice and the deposit was allowed to dry between applications. The entire treatment was repeated twice a week during the period of the spring migration.

Colonisation of the hops in methyl salicylate, β-acid and control plots was assessed by counting the number of alate and apterous damson-hop aphids found on samples of 25 leaves picked at random from the uppermost parts of the hop plants. Counts were made twice-weekly at the start of the migration (19 May to 5 June 1994), then once a week until the spring migration ended.

2.3 Experiments with potential attractants for spring migrants

Treatment E consisted of β-caryophyllene and (*E*)-2-hexanal in a 2 : 78 ratio, which had been identified as the biologically active component of the headspace collected from hop-infested plants.[3]

The steam distillate of hops (**F**) was prepared from freshly cut hop shoots (cv. Emerald) (320 g fresh weight) using standard procedures. (Plant material was macerated with ice-cooled distilled water and subjected to steam distillation. The distillate was extracted with pentane, the resulting solution dried over sodium sulfate and evaporated to dryness, yielding 2 g of a yellow oil.)

Hop headspace volatiles from uninfested young hop plants were obtained by forcing a stream of dry nitrogen through a desiccator containing samples (approximately 700 g) of freshly cut young hop plants (cv. Emerald), the volatiles either being adsorbed on Tenax or collected in a trap cooled with liquid nitrogen.

Samples eluted with methylene chloride (Tenax sample) or dissolved in dioctyl phthalate (cold trap) were analysed using GC-MS. Based on the results of these analyses, synthetic mixtures containing the main components identified were made and used in the traps. Mixture **H** (Tenax) contained 3-pentanone, 2-buten-1-ol (*E,Z* mixture), (*E*)-3-hexen-1-ol, 3-octanone and 1-hexenol in equal amounts. Mixture **K** (cold trap) contained (*E*)-2-hexenal, (*E*)-3-hexen-1-ol, 1-octen-3-ol (racemic mixture), (*E*)-3-hexen-1-ol and (*Z*)-3-hexen-1-ol in the proportions 40 : 30 : 15 : 10 : 5. A further trap was equipped with vials containing (*E*)-3-hexen-1-ol (**G**). Based on GC-MS analyses of a steam distillate fraction isolated from a commercial CO_2-hop extract (Hopfenextraktion HVG, Barth Raiser & Co., Wolnzach), a further synthetic mixture (**L**) containing α-humulene, β-myrcene and β-caryophyllene (40 : 35 : 15) was prepared. Sample **M** consisted of a steam distillate of fresh hop-cones provided by the Hop Research Institute, Hüll.

1-Octen-3-ol was prepared by Grignard-reaction of hexanal with vinyl-magnesium-bromide. All other components were purchased from Aldrich, Sigma or Fluka (purity >95%).

The chemicals (10 mg) were fitted to clear water-traps, chosen in preference to yellow traps for this experiment, to avoid possible interactions with visual cues.

2.4 Pheromones, kairomones and the autumn migrants

The main component of the damson-hop aphid sex pheromone the *cis,cis*-nepetalactol (Fig. 1; structure **1**) was prepared either from naturally occurring *cis,cis*-nepetalactone or by a total synthesis.

2.4.1 Naturally occurring cis,cis *nepetalactone*

Nepeta racemosa (syn. *mussini*) Spreng ex Henckel (Lamiaceae) (5 kg fresh weight of plant material) was steam distilled[2] to yield a crude extract (15 g) containing 95% nepetalactones, 49% of which was the required *cis,cis*-stereoisomer. The crude extract was then separated by column chromatography on silica gel (Merck, Kieselgel 60, particle size 0·040–0·063 nm; eluted with cyclohexane + ethylacetate, 10 + 1 by volume). The resulting pure *cis,cis*-nepetalactone **2** (Fig. 1) was then reduced to the corresponding *cis,cis*-nepetalactol **1** (Fig. 1) using diisobutylaluminium hydride (DIBAH) in dichloromethane. This reaction creates a new stereogenic centre at carbon-1 and leads to a mixture of two diastereoisomers.

A further sample of the crude steam distillate was subjected to the reduction procedure without prior

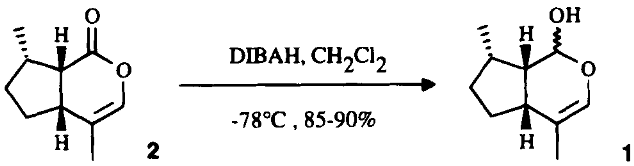

Fig. 1. Reduction of *cis,cis*-nepetalactone (**2**) to *cis,cis*-nepetalactol (**1**), the main component of the *P. humuli* sex pheromone.

purification, giving a mixture of nepetalactols with different stereochemistry (49% *cis,cis*).

2.4.2 Total synthesis

The total synthesis (Fig. 2) was based on the method described by Suemune *et al.*,[11] who synthesised nepetalactones starting from (−)-limonene. The *cis,cis*-nepetalactone with the correct stereochemistry i.e. 4a*R*, 7*S*, 7a*S*, was prepared in eight steps starting with (+)-limonene-oxide **3**. Ring opening of the epoxide ring and periodate cleavage of the diol **4** yielded the unsaturated ketoaldehyde **5**. Such γ,δ-unsaturated aldehydes can be cyclised to cyclopentanones using tristriphenylrhodium-I-chloride as a catalyst, yielding the cyclopentanone **6** with high stereoselectivity. With its two keto groups, the cyclopentanone **6** can easily undergo an intramolecular aldol-condensation under weakly acidic conditions to yield the unsaturated bicyclic ketone **7**. Hydrogenation of **7** led to formation of the saturated bicyclic ketone **8**. Since the attack of the hydrogen proceeded with high stereoselectivity on the stereochemically less hindered side of the convex molecule, the *cis,cis*-isomer was formed. Formation of the silyl enol ether **9**, followed by ozonolysis of the double bond resulted in the formation of the carboxaldehyde **10**, which cyclised under acid conditions, giving rise to the *cis,cis*-nepetalactone **2**,

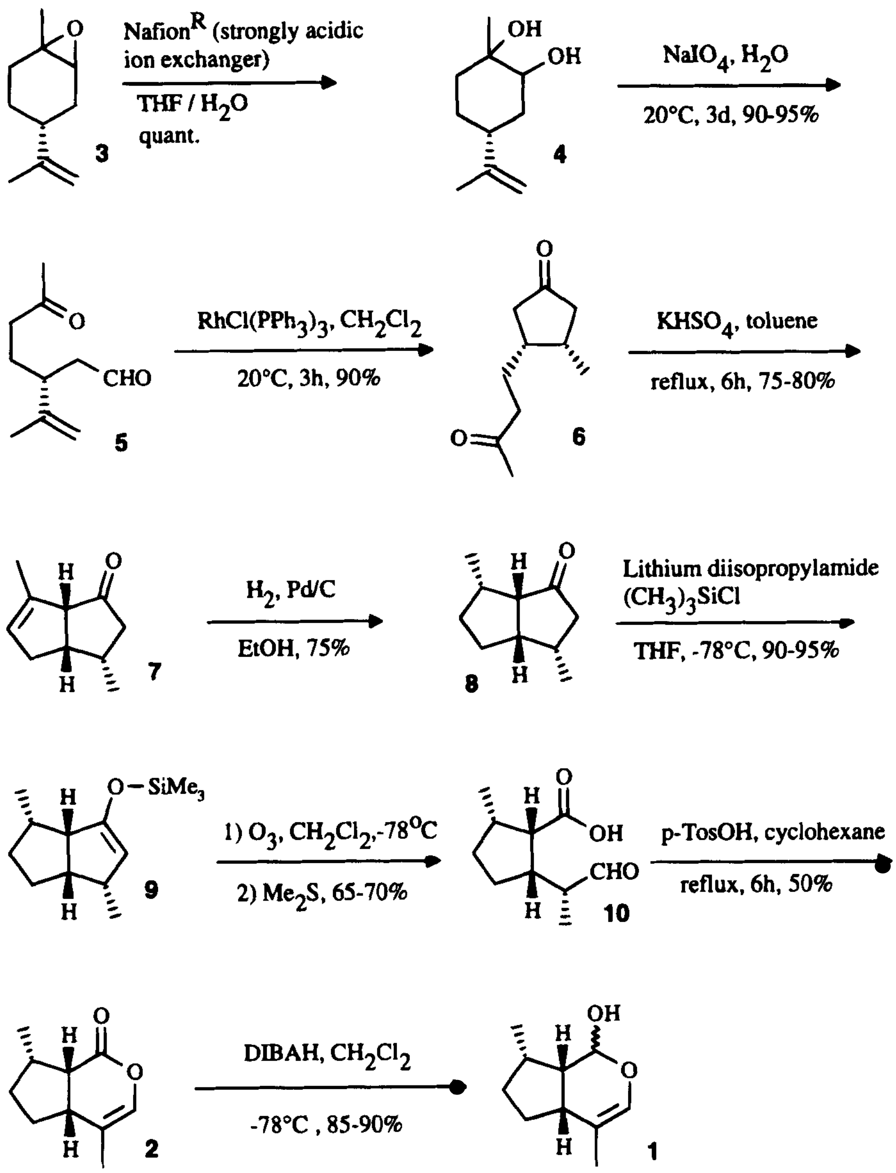

Fig. 2. Schematic representation of the preparative route for the total synthesis of *cis,cis*-nepetalactol (**1**) starting with (−)-limonene.

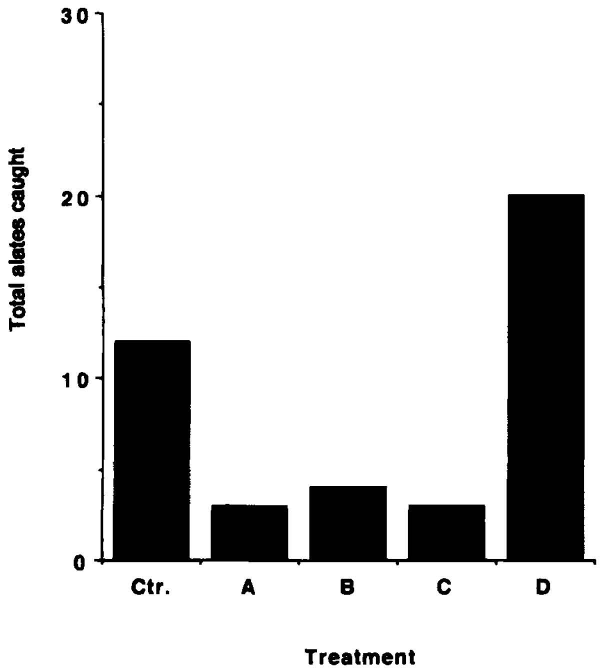

Fig. 3. Effect of repellents on total catches of *P. humuli* in yellow, water-filled traps. (**Ctr**) untreated; (**A**) methyl salicylate; (**B**) butyl isothiocyanate; (**C**) 4-pentenyl isothiocyanate; (**D**) diethyltoluamide.

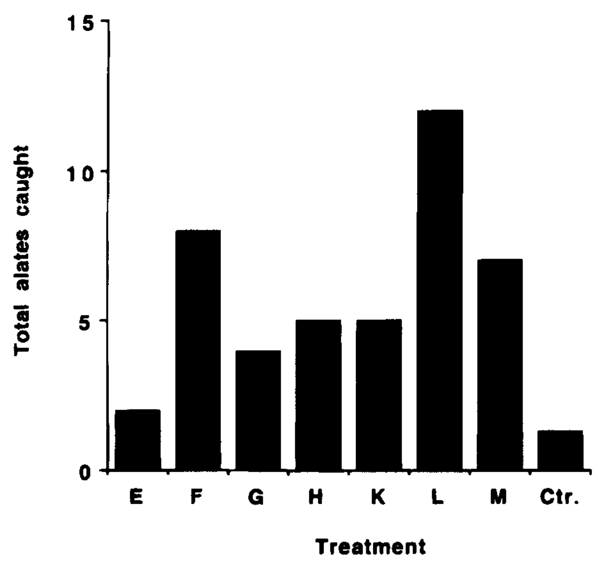

Fig. 4. Effect of *P. humuli* attractants on total catches of spring migrants in clear, water-filled traps. (**Ctr**) untreated; (**E**) β-caryophyllene + (*E*)-2-hexenal (2 + 78); (**F**) steam distillate of young, freshly cut hop shoots; (**G**) (*E*)-3-hexen-1-ol; (**H**) Synthetic headspace based on Tenax adsorption, 3-pentanone + 2-buten-1-ol (*E,Z* mixture) + (*E*)-3-hexen-1-ol + 3-octanone + 1-hexenol in equal amounts; (**K**) Synthetic 'headspace' based on cold-trap sample (*E*)-2-hexenal + (*E*)-3-hexen-1-ol + 1-octen-3-ol + (*E*)-3-hexen-1-ol + *Z*-3-hexen-1-ol (40 + 30 + 15 + 10 + 5). (**L**) Synthetic mixture based on GC-MS analyses of a steam distillate fraction isolated from a commercial CO_2-hop extract (α-humulene + β-myrcene + β-caryophyllene ratio (40 + 35 + 15); (**M**) Steam distillate of fresh hop cones.

which was reduced to the corresponding lactol **1** as described above.

2.4.3 Attractiveness of pheromones

In the experiment comparing the attractiveness of the pheromone preparations made in different ways (pure *cis,cis* or crude extract) all pheromone dispensers were fitted on clear water-traps; however while those containing the synthetic material contained 10 mg of pure *cis,cis* form, those with crude extract contained 20 mg since this preparation contained only 49% of the *cis,cis* form.

2.4.4 Extract of plum leaves

A steam distillate of plum leaves (*Prunus domestica* L.) was tested for its effect as a primary host kairomone. Leaves (1·8 kg fresh weight) were collected in mid-August, as close as possible to the start of the autumn migration, and yielded 0·7 g of the plum-leaf extract. This extract (20 mg) was tested alone and in combination with *cis,cis*-nepetalactol (10 mg), with separate dispensers fitted to both clear and yellow water-traps.

2.4.5 Influence of dose

The influence of dose of the sex pheromone on catches of *P. humuli* gynoparae and males was investigated using clear water-traps. Treatments were *cis,cis*-nepetalactol at six doses (0·001, 0·01, 0·1, 1, 10 and 100 mg) plus three control traps not fitted with pheromone-releasing vials.

2.4.6 Influence of Prunus *kairomones and trap-variables*

In the investigations of the effects of trap height, pheromone dose and interactions between pheromone, kairomones and clear versus yellow-coloured traps, the *cis,cis*-nepetalactol used was obtained by reduction of purified *cis,cis*-nepetalactone from the *N. racemosa* extraction.

2.5 Vertical distribution of damson-hop aphids

Seven delta-traps (Oecos, UK) fitted with a PVC-Vial-Dispenser (WP/5 Fisons) containing *cis,cis*-nepetalactol (10 mg) were set at 1-m intervals above the ground, with the lowest trap at 1 m. The experiment was conducted in the centre of the experimental plot from 24 September 1992 to 2 October 1997. Sticky trap liners were replaced daily. The numbers of *P. humuli* males and gynoparae caught were counted.

3 RESULTS

3.1 Spring migration

3.1.1 Effect of potential repellents

Traps with methyl salicylate and the two isothiocyanates caught significantly fewer aphids than the control ($Chi^2 = 6{\cdot}75; P < 0{\cdot}01$) (Fig. 3). Diethyltoluamide not only failed to deter *P. humuli* from landing in the

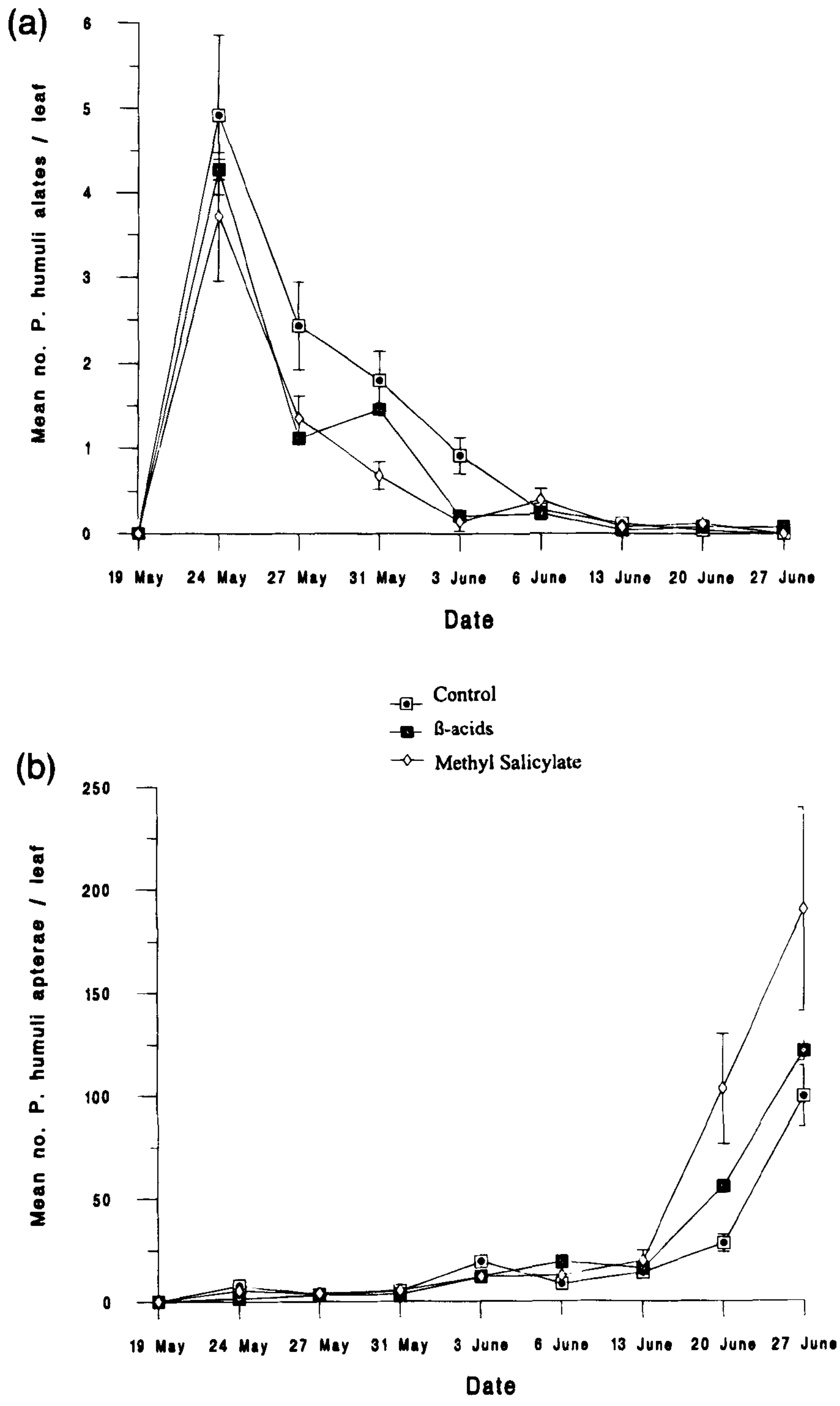

Fig. 5. (a) Colonisation of hops with *P. humuli* spring migrants and (b) development of the apterous population in untreated hops, in hops with slow release 'rope'-formulation of methyl salicylate and in hops sprayed with β-acids. Mean number of aphids on samples of 25 leaves $\pm$ standard error of mean.

yellow traps, but attracted a greater total of aphids than did the control ($Chi^2 = 5{\cdot}37$; df 1; $P < 0{\cdot}05$).

3.1.2 *Effect of potential attractants*

Catches were generally low in the clear traps (Fig. 4). With the exception of treatment E, which released the caryophyllene/hexenal mixture, catches for all treatments lay significantly above the level of the control (Chi^2 test; $P < 0{\cdot}05$). In the case of treatments **L**, **F** and **M**, which released volatiles from the synthetic mixture of terpenes based on analyses of a commercial CO_2-hop cone extract, from steam-distilled young hop shoots, and from steam-distilled fresh hop cones, respectively,

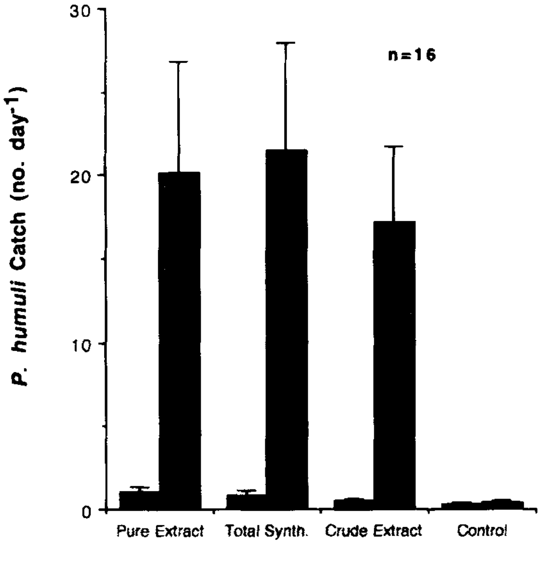

Fig. 6. Effect of preparative route on the attractiveness of samples of *cis,cis*-nepetalactol to *P. humuli* males and gynoparae caught in clear water-traps (Mean ± standard error of mean; Solid bars = Gynoparae, hatched bars = males).

the difference compared to the control was greatest and statistically highly significant ($P < 0{\cdot}001$). Mixtures based on the main components found in Tenax and cold-trap headspace fractions collected from young, uninfested hops showed catches intermediate between those from the control and other treatments.

3.1.3 Tests with potential repellents

The curves for mean numbers of spring migrant *P. humuli* per leaf on untreated hops and on those treated with the formulation of methyl salicylate and an aqueous spray of β-acids followed similar courses (Fig. 5(a)). The maximum number of alate *P. humuli* was recorded on 24 May 1994 in all plots. Thereafter, numbers landing decreased rapidly with values of less than one alate per leaf recorded from 3 June until the end of the experiment. Although the mean number of alates found on untreated hops was consistently higher than in the methyl salicylate-treated plot, analysis of normalised data for the period 24 April to 20 June revealed that this difference was not statistically significant ($t = 1{\cdot}55$; df 12; $P > 0{\cdot}05$). In the case of β-acid-treated hops, owing to a generally smaller spread in the numbers of alates found on leaves sampled, the suppression of aphid numbers compared to the control was significant ($t = 2{\cdot}29$; df 12; $P < 0{\cdot}05$).

The curves for the development of apterous *P. humuli* populations in the control and in both treated plots (Fig. 5(b)) show a slow rise between the start of the experiment and 13 June, during which period there was no significant difference between treatment and control plots. This was followed by a phase of rapid population growth in all plots from 20 to 27 June. During this latter period, the aphid numbers in the methyl salicylate-treated plot were significantly greater ($t = 4{\cdot}11$; df 4; $P < 0{\cdot}02$) than in the control. Over the same period the β-acid treatment had no significant effect on apterous aphid numbers when compared to the control ($t = 2{\cdot}02$; df 4; $P > 0{\cdot}05$).

3.2 Autumn migration

3.2.1 Effect of mode of preparation of cis,cis-*nepetalactol*

A comparison of mean numbers of male *P. humuli* caught daily in clear water-traps with *cis,cis*-nepetalactol derived from the three preparative routes showed no

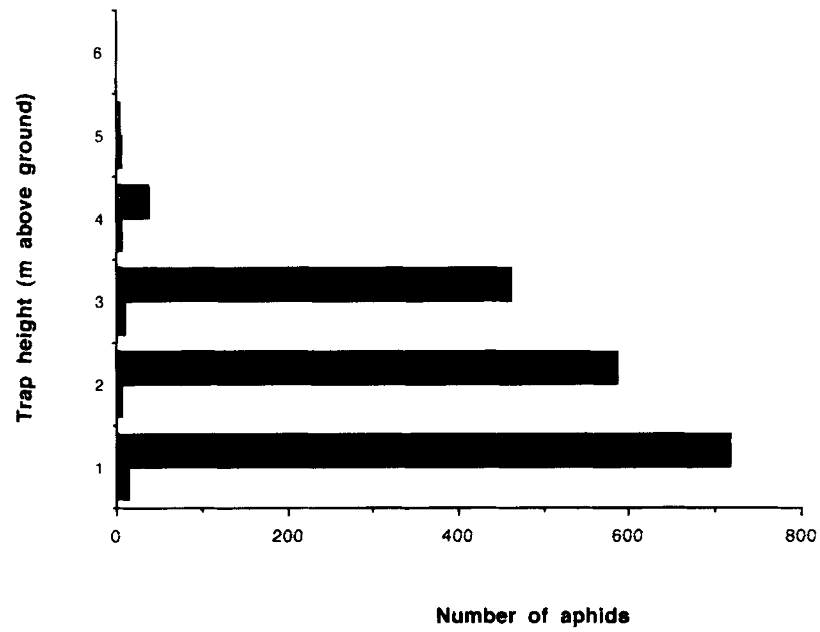

Fig. 7. *P. humuli* males and gynoparae caught in delta traps suspended at different heights inside the hop garden between 24 September and 2 October 1992 (Solid bars = gynoparae; hatched bars = males).

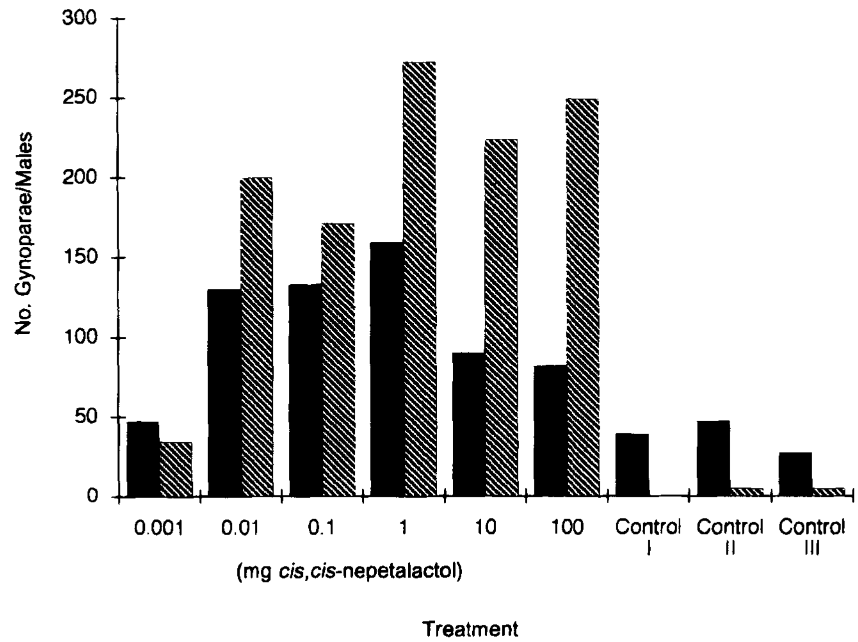

Fig. 8. Effect of *cis,cis*-nepetalactol dose on catches of *P. humuli* males and gynoparae in clear water-traps (solid bars = gynoparae; hatched bars = males).

significant difference in their relative attractiveness. They all caught significantly more males ($P < 0{\cdot}001$) than did the control trap without pheromone (Fig. 6). As the experiment was conducted in the later part of *P. humuli*'s autumn migration, few gynoparae were trapped and the mean numbers caught in the pheromone treatments were not significantly different from that in the control.

3.2.2 Effect of trap height on catches of gynoparae and males

Most males were caught in traps at lower heights (Fig. 7), despite the high densities of aphids on foliage at all heights up to 7 m. Statistically, this observed distribution of aphids differed significantly from that expected assuming an even distribution of *P. humuli* at all heights in the hop garden ($\text{Chi}^2 = 160$; df. 6; $P < 0{\cdot}001$). Since the experiment was done during late autumn when the migration of gynoparae had largely ended, few individuals of this morph were caught, and no clear trend emerged.

3.2.3 Effect of cis,cis-*nepetalactol dose on pheromone-trap efficacy*

Catches of both males and gynoparae showed similar trends of increased catch rates with increasing amounts of nepetalactol load over the 1 to 1000 μg range (Fig. 8). However, traps fitted with dispensers containing 10 or 100 mg nepetalactol caught successively fewer gynoparae (Chi^2 for gynopara catch in 1 mg versus 100 mg traps = 77; df 1; $P < 0{\cdot}001$). In contrast, catches of males remained at around the maximal level achieved with a 1-mg load of nepetalactol over the same range of high doses (Chi^2 for male catches with 1 mg versus 100 mg nepetalactol = 1·94; df 1; $P > 0{\cdot}05$).

3.2.4 Interaction of pheromone, winter-host kairomones and trap colour

Both males and gynoparae were most numerous in yellow traps with both pheromone and plum kairomones (Fig. 9). Comparison of these catches with those in clear traps with dispensers containing the same volatiles showed that, for both morphs, the presence of the colour stimulus more than doubled the catches. The

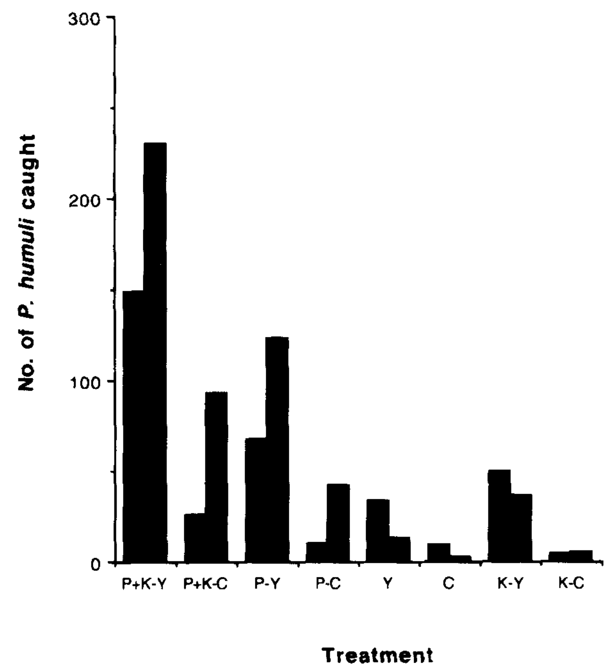

Fig. 9. Differential catches of *P. humuli* with yellow and clear traps incorporating primary host kairomones and *cis,cis*-nepetalactol (sum of six days' results). **(P)** *cis,cis*-nepetalactol (10 mg), **(K)** plum-leaf steam distillate (20 mg), combined with yellow traps (-Y) and clear traps (-C). (solid bars = gynoparae; hatched bars = males).

experiment was conducted approximately mid-way through the *P. humuli* flight period when gynoparae were generally more numerous than males.

4 DISCUSSION

4.1 Spring migration

4.1.1 Headspace volatiles

Analyses of headspace samples collected by the various methods described showed that the trapping system employed (cold trapping, adsorption on Tenax) had a major effect on the profile of the identified headspace components. Thus substances identified in the cold-trapping sample and that from Tenax adsorption were for the most part typical 'green-leaf volatiles' (e.g. different hexenols). When tested in the field, they had some effect on aphid catches in clear water-filled traps, but were generally less effective than either of the steam distillates derived from various sources of hop plant material. Somewhat surprisingly, the effect of the caryophyllene/hexenal mixture, based on GC-SCR analyses of Porapak-entrained headspace volatiles collected from *P. humuli*-infested plants, was disappointing when tested in the field. These results contrast with positive results obtained in laboratory bioassays.[3]

The very low total catches of spring migrants in clear Petri-dish traps provided with vials containing plant chemicals contrasts markedly with autumn-migrant catches in identical clear traps with vials releasing the sex pheromone. The fact that the 'best' kairomone treatment tested in a clear trap in this experiment only just equalled the catch total of the yellow control trap in the trapping experiment with repellents demonstrates how important the correct visual cues are in host-orientation behaviour by spring-migrant damson-hop aphids.

4.1.2 Methyl salicylate

The results of trapping experiments with methyl salicylate, a component from the hop headspace, and the isothiocyanates which are found in the headspace of certain non-host plants (e.g. *Brassica* spp. (Brassicaceae)), demonstrate the breadth of chemical cues from their environment that damson-hop aphids are able to perceive and that they may be using in the process of host-plant selection. The repellent effect of methyl salicylate indicated by reduced trap catches demonstrates that by no means all components of the hop headspace must necessarily have an attractive (i.e. kairomonal) effect and underlines the important role played by plant secondary metabolites in host plant defence.[7,8] In the experiment where a slow release formulation of methyl salicylate was applied to hops, the intention was therefore to augment the concentration of this allomone in the 'bouquet' of headspace volatiles so that, from a distance, the treated plants might appear less attractive to *P. humuli* and be avoided. No significant reduction was recorded in the number of migrant *P. humuli* colonising the crop, although the data suggest that the treatment may have had a slight repellent effect. However, significantly higher population densities of the economically damaging apterous form of the hop-aphid developed on the methyl salicylate-treated plants. For plants treated with potential repellents to be more heavily infested with apterous damson-hop aphids than the untreated controls is surprising and warrants further investigation. Possibly natural enemies were adversely affected by methyl salicylate, allowing the aphid population to develop under reduced pressure of predation. In an integrated control strategy, in which predation by beneficial arthropods would play a key part, the timing of repellent treatments might therefore have to be considered carefully. Evidently, any repellent effect of methyl salicylate, even in the form of a high-release-rate rope formulation containing the maximum possible amount of active material (3% by volume) is too weak to counter the combined attractive effect of other stimuli from the hops (kairomones, shape, colour).

4.1.3 β-acids

Although repeated treatment of hops with hop resin containing β-acids did have a measurable repellent effect on spring migrants of *P. humuli*, this was too weak to be of practical value. Colonisation occurs during the period of the hop's maximal growth (up to 20 cm per day), so that, even with two treatments per week, it is virtually impossible to ensure the complete coverage of new growth with a contact repellent. Since it is precisely these parts of the plant which form the preferred site for *P. humuli* spring migrants to deposit their larvae, the use of surface-applied, non-volatile contact repellents is probably not viable as an approach for protecting young hop plants against colonisation by *P. humuli*.

4.2 Autumn migration

The efficacy of *cis,cis*-nepetalactol as an attractant for male *P. humuli* and its additional, but weaker, action as an aggregation pheromone for gynoparae of this species have been described elsewhere,[9,10] and were confirmed here. The relatively small difference that the method of preparation, and consequently the stereochemical purity, of the *cis,cis*-nepetalactol, had on its attractiveness to male damson-hop aphids suggests that the presence of other nepetalactol isomers does not interfere with sex-pheromone-mediated behaviour in this species. By contrast, the amount of pheromone dispensed in the traps had a strong influence on catches. The fact that higher pheromone dosages had a deleterious effect on catches of gynoparae and not males may indicate that large concentrations of pheromone in the air may confuse gynoparae. A high pheromone concentration in nature would be indicative of the presence of many oviparous females. It would be interesting to investigate

whether gynoparae show a behavioural reaction to the spacing of oviparous females on the winter host plant, where there may be a trade-off between, on the one hand, the presence of a certain number of oviparae indicating a high quality winter host, and, on the other hand, high densities of oviparae indicating competition for mates, egg-laying sites or other limited resources.

Lösel *et al.*[9] showed that the simultaneous presentation of volatiles of the winter host with *cis,cis*-nepetalactol enhanced catches of both males and gynoparae, and this was confirmed here. The results of this study, however, show that the effects of the pheromone and kairomone cues can be further enhanced by using a yellow trap to provide a visual stimulus. This finding agrees with similar observations for a number of different aphid species.[10]

The height above ground level at which pheromone traps were placed also had a marked effect on catches of males. Possibly, this may be attributable to variations in wind speed and turbulence affecting male aphids' ability to orientate and manoeuvre towards traps. A practical consequence is that traps for both monitoring and controlling this aphid in the autumn, as well as applications of lure and kill formulations, should be placed at around 1 m above the ground for maximum efficacy.

The comparatively low catches for both gynoparae and males in clear traps with pheromone alone, and in yellow traps with no odour source, suggests that visual and pheromonal cues are complementary. For the primary host kairomones, a similar, albeit weaker, behavioural interaction also appears to be taking place, with catches in the 'yellow trap-plus-kairomone' combination being greater than the sum of catches in clear trap-kairomone and simple yellow traps.

4.3 Semiochemicals as crop-protection agents for hop growers?

While the results of the above field experiments show that certain simple compounds, as well as relatively simple mixtures, can exert a behaviour-modifying effect on *P. humuli*, the extent to which this occurs varies enormously according to the class of substance (kairomones or sex pheromones) and the aphid morph involved. Thus, while a number of the chemicals tested had demonstrable effects on spring migrants in the field, these were, as they stand, insufficiently strong to form the basis of a crop-protection strategy. Further work is therefore needed to clarify the role of different identified components of the hop headspace odours in the process of host-plant selection by *P. humuli*. Of crucial importance will also be the optimisation of the release rate of one or several such components. Offered at too low a concentration, it is unlikely that their activity will be sufficient to 'compete' with the natural source. Equally, release of an unnaturally high concentration may cause the same substances to induce different behavioural activities.

In terms of the strength of the behaviour-modifying effects observed, the combination of olfactory and visual stimuli tested in the autumn looks more promising. The aim would be to control the overwintering population through trapping males, and also gynoparae if possible, within the hop garden and before they could reach the primary host plant. Because of the relatively small area of ground covered by individual hop gardens, a 'mating confusion' approach would seem less appropriate than a trapping approach (either mass trapping or lure-and-kill). From a commercial view point, however, a strategy centred on the autumn migration is much harder to visualise in practice. Being preventative it would have to be carried out in a coordinated fashion, involving all hop growers in a region, for example, and possibly need repeating for several years before significant effects showed.

A point in urgent need of further clarification is the distance over which migrating damson-hop aphids contribute significant numbers to infestations at particular sites. A prophylactic sex-pheromone-based strategy would probably be most effective where the majority of the migrating population originates from within a short distance of the treated area, and where the potential for populations of *P. humuli* to survive on wild hops within the treatment area could be eliminated through appropriate cultural practices. As an approach directed towards disruption of the sexual phase of reproduction, in contrast to conventional aphicidal treatments aimed at combating the asexual stages of the aphids' life cycle, it would be interesting to consider if such a prophylactic approach might be used as a tool in insecticide resistance management. Trapping any remnants of the damson-hop aphid population surviving a conventional treatment might counter the spread of insecticide-resistant genotypes back into the wild population, which ultimately represents the reservoir from which the renewed infestation will emerge in the following hop-growing season.

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